

ToxinSensor™ Gel Clot Endotoxin Assay Kit

Cat. No. L00351

Technical Manual No. 0356

Version 05312018

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I DESCRIPTION

GenScript **ToxinSensor™ Gel Clot Endotoxin Assay Kit** is designed as a simple and sensitive *in vitro* end-product endotoxin test for human and animal parenteral drugs, biological products, and medical devices. The Limulus Amebocyte Lysate (LAL) test is a qualitative test for Gram-negative bacterial endotoxin. Limulus Amebocyte Lysate supplied in the kit needs to be reconstituted with LAL Reagent Water and then mixed in equal parts with the solution being tested. After incubation, and in the presence of endotoxin, gelation occurs; in the absence of endotoxin, gelation does not occur. The kit contains all the necessary reagents and endotoxin-free materials. The sensitivity of LAL in this kit is 0.25 EU/ml. The protocol described herein conforms to those described in the FDA guideline. Similar performance requirements for gel clot assays have been published and are updated regularly in the United States Pharmacopeia.

II KIT CONTENTS

Components	L00351
Size	40 Assays
Limulus Amebocyte Lysate (LAL), 2 ml/vial	2 vial
LAL Reagent Water, 10 ml/bottle	4 bottles
<i>E. coli</i> Endotoxin Standard	2 vials
Endotoxin-free vial	5 × 17
Endotoxin-free Tips, 200 µl	1 box (96 tips)
Endotoxin-free Tips, 1000 µl	2 bags (12 tips)
Incubation Rack	1

III MATERIALS AND EQUIPMENT NOT PROVIDED

1. Sodium hydroxide, 0.1 N dissolved in LAL Reagent Water, for pH adjustment.
2. Hydrochloric acid, 0.1 N dissolved in LAL Reagent Water, for pH adjustment.
3. Oven or non-circulating hot water bath ($37 \pm 1^\circ\text{C}$)
4. Test tube rack.
5. Vortexer.

IV STORAGE

The kit can be stored dry at room temperature for up to one month. For long-term use, the kit can be kept at $2-8^\circ\text{C}$ for up to 24 months. Do not freeze the kit or any of its components.

V ENDOTOXIN DETECTION PROTOCOL

1. Specimen Preparation

All materials or diluents used for specimen collection and test reagent preparation must be endotoxin-free. Use aseptic technique at all times. Samples to be tested must be stored in such a way that all bacteriological activity is stopped or the endotoxin level may increase over time. For example, samples can be stored at $2-8^\circ\text{C}$ within 24 hours before use, but need to be stored frozen if not used within 24 hours.

Since the LAL-endotoxin reaction is pH dependent, it may be necessary to adjust the pH of the sample to within the range 6.0-8.0 using endotoxin-free sodium hydroxide or hydrochloric acid. Always measure the pH of an aliquot of the bulk sample to avoid contamination by the pH electrode.

Dissolve or dilute test specimen using LAL Reagent Water. It may be necessary to determine the degree of dilution by calculating the MVD value. Maximum Valid Dilution (MVD) is equal to the expected maximum endotoxin concentration in the test sample divided by lambda. Lambda is labeled lysate sensitivity of endotoxin standard. In this kit, lambda is 0.25 EU/ml.

Note: The specimen should be certified free of Beta Glucans contaminant which may come from yeast and cellulosic materials, such as blood products.

2. Reagent Preparation

Limulus Amebocyte Lysate (LAL)

Reconstitute lyophilized lysate by adding 2 ml LAL Reagent Water to the vial. Swirl gently for at least 30 seconds to

thoroughly dissolve the lysate. Do not shake or vortex to avoid foaming. Reconstituted lysate can be stored at -20°C or below for up to one week if frozen immediately after reconstitution. Avoid repeated freeze and thaw cycles.

***E. coli* Endotoxin Standard**

Dissolve lyophilized endotoxin standard by adding 1 ml of LAL Reagent Water. Mix thoroughly for at least 15 minutes with a vortexer to obtain an endotoxin stock solution. Prepare 0.5 EU/ml endotoxin solution as positive control. Reconstituted endotoxin standard can be stored at -20°C or below for up to 15 days.

3. Test Procedure

Each assay should include both a positive control and a negative control. LAL Reagent Water can be used as a negative control.

- 1) Carefully dispense 0.1 ml of LAL reagent into the endotoxin-free vials. Label them as negative control, positive control and sample, respectively.
- 2) Carefully transfer 0.1 ml of positive control, negative control and the test samples to the LAL reagent in step (1). Cap the vials and mix them thoroughly.
- 3) Place all the vials in the incubation rack and incubate the vials at $37 \pm 1^\circ\text{C}$ by placing the rack in a non-circulating hot water or oven.
- 4) Remove the rack after 60 ± 2 minutes of incubation, invert each vial and check whether a gel is formed or not.
 - a) A positive reaction is characterized by the formation of a firm gel that remains intact when the vial is inverted.
 - b) A negative reaction is characterized by the absence of a solid clot. The lysate may show an increased turbidity or viscosity. This is considered a negative result.
- 5) Calculation of endotoxin level. In this test, the endotoxin level in the positive sample is equal to or higher than 0.25 EU/ml; while in the negative sample, the endotoxin level is lower than 0.25 EU/ml.

4. Application Example

- 1) Sample: Protein A (1 mg/ml in PBS, pH 7.4) purified from recombinant *E. coli* lysate using Ni-NTA Resin.
- 2) Dilutions using LAL Reagent Water: 1: 200,000, 1: 400,000, 1: 800,000.

The test is performed as the procedure above and the assay result is shown in the table below,

Positive control	Negative control	1: 200,000	1: 400,000	1: 800,000
+	-	+	-	-

- 3) Endotoxin concentration in this sample is, therefore, between 50,000 and 100,000 EU/ml.

VI ORDERING INFORMATION

Product Name	Cat. No.
ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit (32 rxns)	L00350
ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit (16 rxns)	L00350C
ToxinSensor™ Gel Clot Endotoxin Assay kit	L00351
ToxinEraser™ Endotoxin Removal kit	L00338

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